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Urinary excretion rate of endothelin-1 in patients with essential hypertension and salt sensitivity

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Urinary excretion rate of endothelin-1 in patients with essential hypertension and salt sensitivity. To assess the possible role of ET-1 in the pathogenesis of hypertension and salt sensitivity levels of immunoreactive endothelin-1 (irET-1) were measured in plasma and urine of 17 patients with essential hypertension and in 19 normotensive control subjects. Effects of alterations in dietary sodium content on urinary irET-1 levels also were assessed. Plasma levels of irET-1 did not differ between the hypertensives and normotensive groups (1.1 ± 0.3 and 1.3 ± 0.1 pg/ml). Urine samples of both groups contained high concentrations of irET-1. However, the mean daily urinary excretion of irET-1 in the hypertensives was less than one-third that in controls (29 ± 3 vs. 109 ± 21 ng/day, respectively, $P < 0.01$). Changing dietary sodium content in the hypertensives had no effect on mean irET-1 excretion. However, on either low, intermediate, or high salt diet, "salt sensitive" hypertensives had lower levels of the peptide than "salt resistant" patients (23 ± 3 vs. 36 ± 5 ng/day, respectively, $P < 0.05$). The data demonstrate a marked reduction in irET-1 excretion in patients with essential hypertension, despite normal plasma levels of the peptide. Since ET-1 has diuretic and natriuretic properties, the decreased renal excretion of ET-1 may be of relevance to the pathophysiology of hypertension and salt sensitivity.

The endothelins (ET) are a new family of biologically active peptides that were first detected in the media of cultured porcine endothelial cells [1]. They are potent vasoconstrictors when tested on isolated arteries and veins *in vitro* [1], and cause a uniquely prolonged increase in arterial blood pressure when given in pharmacological doses *in vivo* [1, 2]. Three isoforms of ET are encoded by the human genome and the most potent one, ET-1, has been demonstrated to exist in human vascular endothelial cells [3]. Because of their powerful effects on vascular smooth muscle cells and their location in endothelial cells, they have been suggested to participate in the physiologic regulation of systemic blood pressure and regional blood flow [1, 3].

The vasoactive properties of ET make them possible candidates for involvement in the pathogenesis of essential hypertension (EHT). Indeed, Saito et al have recently reported increased plasma levels of ET-1 in patients with EHT [4], suggesting a causative role for ET-1 in the pathogenesis of the disease. However, Davenport et al [5], using different analytical methods, failed to detect any significant increase in plasma ET-1 levels in his hypertensive patients. Similarly, Suzuki et al [6], found no increase in plasma ET-1 levels in experimental hypertension in rats.

To assess the possible role of ET-1 in the pathogenesis of hypertension and salt sensitivity we measured the concentrations of the peptide in plasma of patients with uncomplicated essential hypertension and compared them to those of normal controls. In addition, since ET-1 levels in plasma may not reflect the activity at the tissue level, and since urinary ET-1 excretion has been suggested to serve as a measure of total ET-1 production [7], we also determined ET-1 levels in the urine of these subjects. Furthermore, since sodium intake has a marked effect on renal vascular reactivity to exogenous ET-1 in experimental hypertension [8], the effect of manipulations in dietary sodium on urinary ET-1 excretion also was investigated.

Methods

Subjects

Seventeen patients (8 men, 9 women, 12 Caucasians, 5 Blacks) previously diagnosed in our clinic as having uncomplicated essential hypertension were studied. Nineteen normal volunteers (7 men, 12 women, 16 Caucasians, 3 Blacks) with no known hypertensive, or any other disease, served as controls. All patients stopped medications two weeks prior to the study. For the assessment of basal excretion rate of ET-1 on a regular diet, a 24-hour urine collection and a fasting venous blood sample were obtained in the morning upon entering the study. Blood samples were immediately separated and the plasma stored at −70°C until assayed.

Salt loading

For the assessment of the effect of sodium intake on urinary ET-1 excretion the 17 hypertensive patients were evaluated during hospitalization at the Clinical Center of the NIH at Bethesda, Maryland, USA. They were allowed a regular diet (normal salt) for eight days, then given a low sodium diet containing 9 mEq/day of NaCl for eight days, followed by a high salt diet containing 240 mEq/day, in the form of salt in tablets, for another eight days. Compliance with the prescribed sodium

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diet was verified in each patient by daily urinary sodium determinations. Mean blood pressure was determined by averaging three consecutive measurements three times daily. Daily urine output was collected in plastic bottles containing 30 ml of 6 N hydrochloric acid. Urine samples for ET-1 determination on either low or high salt diets were obtained on the last day of the corresponding diet. Aliquotes of 2 ml were frozen and stored at −70°C until assayed.

High-performance liquid chromatography

Radioimmunoassay-coupled high-performance liquid chromatography (HPLC) was performed on urine to identify the molecular forms of ET in the urine. Urine was applied to a C_{18} column (μBondapak 3.9 × 300 mm, Waters, Millford, Massachusetts, USA) and ET eluted with a linear gradient of 10% to 60% acetonitrile in 0.1% trifluoroacetic acid (TFA) over 40 minutes, at a flow rate of 1 ml/min. ET-like immunoreactivity was measured in each one minute fraction. Standards of synthetic isoforms ET-1, ET-2, ET-3 and Big-endothelin (1-38) were run on the same column only after urine samples were done.

Radioimmunoassay for ET-1

Immunoreactive ET-1 (irET-1) was measured in plasma and urine samples that were extracted on Sep-Pak C18 cartridges (Waters Associates). The samples were acidified with an equal volume of 0.1% TFA and passed over cartridges activated by acetonitrile. The peptides were eluted with 60% acetonitrile in 0.1% TFA and dried in a vacuum concentrator. The recovery of ET-1 through the extraction phase, as measured by adding labeled ET-1 to each sample, was 85 to 92% for both the hypertensives and the control groups. The reconstituted samples (100 μl) were assayed by a sensitive radioimmunoassay method using a commercially available kit (Peninsula laboratories, Belmont, California, USA) as previously described [9]. IC50 was 18 pg/tube. According to studies done by the manufacturer, cross reactivity with ET-2 and ET-3 was 7% and with Big-ET 35%. Cross reactivity with unrelated peptides (ANP, BNP, angiotensin, vasopressin and VIP) was essentially 0%. Intra-assay and inter-assay coefficients of variance were 7% and 18%, respectively. A dilution curve of urine was roughly parallel to the standard curve (Fig. 1). ET-1 was stable in urine for at least 24 hours in both controls and hypertensives [10].

Statistical analysis

The data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using Student's *t*-test and analysis of variance (ANOVA) were appropriate. All statistical computations were done by a statistical package (StatView 512+). A *P* value of less than 0.05 was considered to represent a statistically significant difference.

Results

There were no significant differences between the hypertensive and the normotensive groups in gender, race, urinary output, or sodium excretion. The average age was 52 ± 4 in the hypertensives and 45 ± 3 in the controls (P = NS). Mean arterial blood pressure (MAP) averaged 107 ± 4 mm Hg in the hypertensives and 91 ± 2 mm Hg in the controls ($P < 0.01$). Creatinine clearance was within normal limits in all hypertensives (mean 96 ± 9 ml/min). Of the control subjects, 11 had a positive family history for hypertension in one or more first degree relatives.

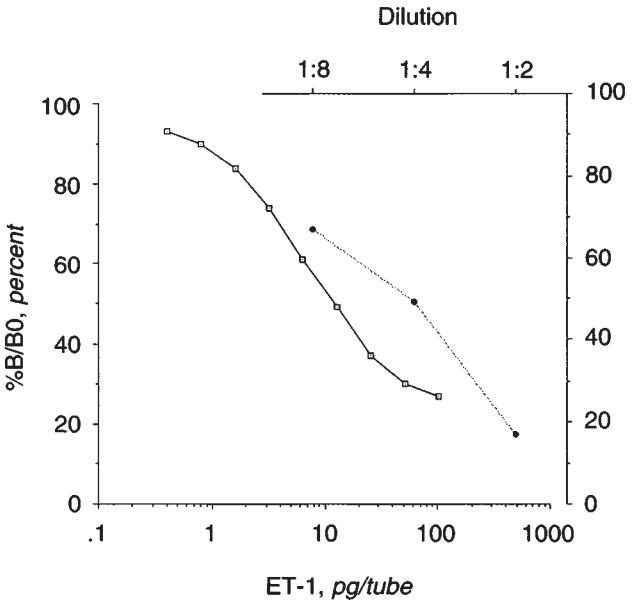

Fig. 1. *Radioimmunoassay for ET-1.* Standard curve used for irET-1 determinations in plasma and urine. A urine sample (··●··) was serially diluted and compared to the standard curve (—⊡—). B/B0- Specific binding/Total binding.

irET-1 concentrations in plasma were very low, near the limit of detection, in both the hypertensive and normotensive control groups. None of the hypertensive patients had an increased plasma level of ET-1. The mean plasma concentration of irET-1 in the hypertensives was 1.3 ± 0.1 pg/ml, and in the controls 1.1 ± 0.3 pg/ml (P = NS).

Reverse-phase HPLC profile of urine is shown in Figure 2. ET-1-like immunoreactivity eluted as a relatively wide major peak corresponding to the retention time of synthetic ET-1. A small amount of irET-1 appeared at an early phase near the solvent front, but no irET-1 was detected at the position of the precursor of ET-1, Big-ET, or at any other position. This indicates that the major component of urinary irET is authentic ET-1.

In contrast to the very low levels in plasma, irET-1 was present in high concentrations in all urine samples in both groups of patients on all types of diet. In the normal controls there was a marked variability in urinary irET-1 levels, the mean irET-1 concentration being 62 ± 11 pg/ml and the daily excretion rate 109 ± 21 ng/day.

Surprisingly, opposite to the predicted increased levels, and in contrast to the normal levels of irET-1 in the plasma, the hypertensive patients had a markedly decreased excretion rate of the peptide into the urine, compared to the normal controls (29 ± 3 ng/day, $P < 0.001$, Fig. 3).

There were no significant correlations between daily irET-1 excretion and either age, gender, race, urinary output, sodium excretion or creatinine clearance ($r = 0.1$, P = NS). Similarly, there was no correlation between daily irET-1 excretion and blood pressure within each group. However, when data from

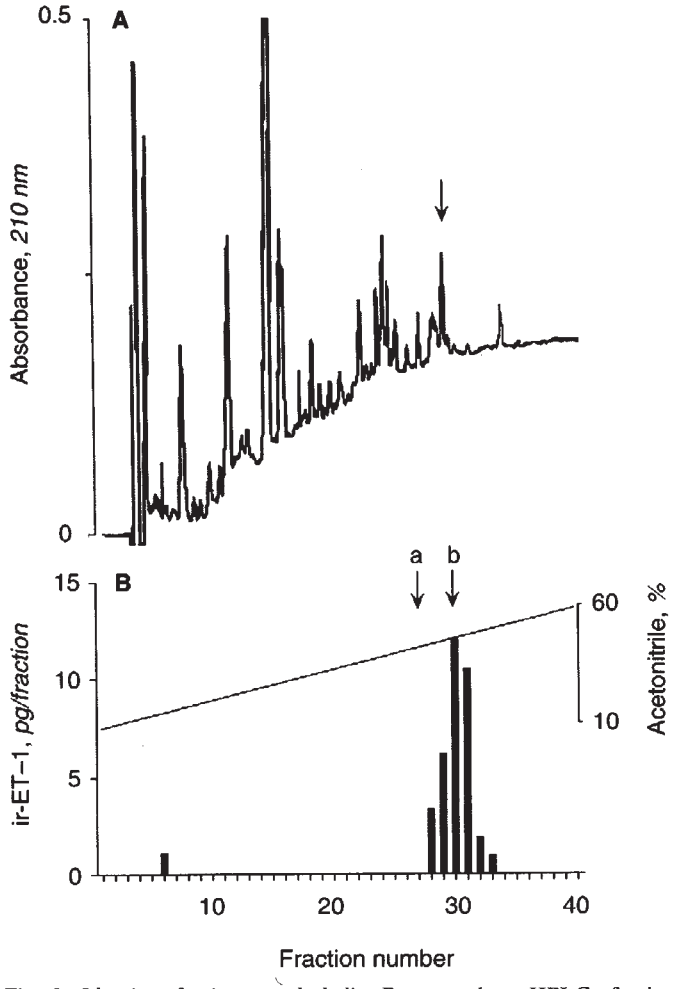

Fig. 2. *Identity of urinary endothelin.* Reverse-phase HPLC of urine (**A**), coupled with RIA for each 1 minute fraction (**B**) reveals a wide major peak corresponding to the retention time of synthetic ET-1 (arrow b). No irET was detectable at the position of Big-ET (arrow a). Retention times of ET-2 and ET-3 were almost indistinguishable from ET-1.

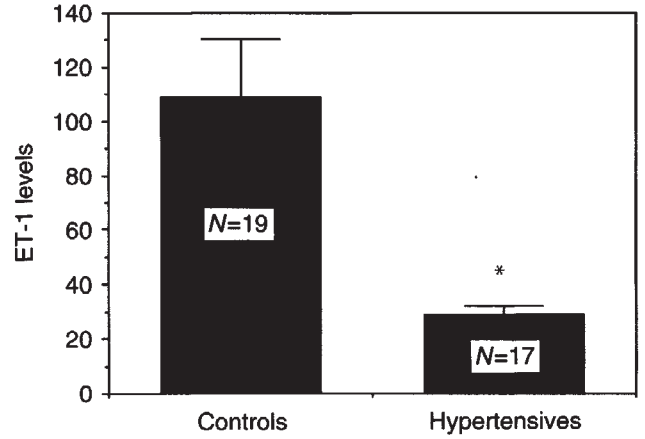

Fig. 3. *Daily urinary excretion rate (ng/day) of irET-1 in normal controls and in patients with essential hypertension.* $*P < 0.01$ for hypertensives vs. controls.

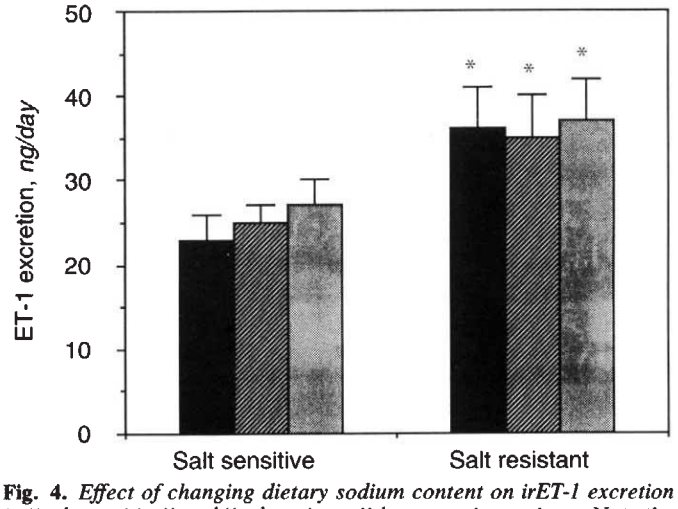

Fig. 4. *Effect of changing dietary sodium content on irET-1 excretion in "salt sensitive" and "salt resistant" hypertensive patients.* Note the significant difference between the two groups on all types of diets. Symbols are: (■) intermediate (normal) salt diet; (▨) low and (▩) high salt diets, respectively. $*P < 0.05$ for salt sensitive vs. salt resistant hypertensives.

control subjects and hypertensives were grouped together there was a weak negative correlation between MAP and daily urinary irET-1 excretion ($r = -0.41$, $P = 0.01$). No correlation was found between a family history of hypertension and urinary irET-1 excretion rate. Likewise, no correlation was found between plasma renin activity and urinary irET-1. Moreover, changing dietary sodium, which had marked effects on PRA (data not shown), had no significant effect on ET-1 levels.

The hypertensive patients were further subdivided into two groups according to their blood pressure response to salt loading [11]. When changed from a low sodium to a high sodium diet, the MAP in a subset of 10 "salt sensitive" hypertensive patients increased more than 8 mm Hg (mean $\Delta MAP = +12$ mm Hg). In the other seven "salt resistant" hypertensives MAP increased less than 8 mm Hg (mean $\Delta MAP = +1$ mm Hg). MAP while on either normal salt diet or on low salt diet were similar in the two subgroups (105 ± 2 and 106 ± 4 mm Hg, respectively).

The effect of changing the salt content in the diet on urinary irET excretion in these two subgroups is shown in Figure 4. The amount of dietary sodium had no significant effect on daily irET-1 excretion in any of the patients. However, the rate of urinary irET-1 excretion in "salt sensitive" patients was significantly lower than in "Salt resistant" patients, regardless of salt intake (average 23 ± 3 vs. 36 ± 5 ng/day, respectively, $P < 0.05$).

Discussion

In this work we studied whether ET-1 may have a role in the pathogenesis of hypertension and salt sensitivity by measuring the concentrations of irET-1 in both plasma and urine, and by assessing the effect of sodium intake on urinary irET-1 levels in patients with EHT. Our results support those previous reports that circulating plasma levels of irET-1 are very low in humans, in the range of 1 to 2 pg/ml, and are not increased in patients with essential hypertension [5, 6]. The reportedly increased

plasma levels in hypertensive patients in the study of Saito et al [4], to the range of 30 pg/ml, may be due to factors like patient selection (that is, including patients with target organ damage), or to other methodological differences. It should be noted that the levels in normal controls, reported in the latter study are more than 10 times higher than the levels obtained by other investigators, possibly due to low specificity of the assay.

In sharp contrast to the normal plasma levels, our cohort of hypertensive patients, had markedly decreased urine levels of irET-1 compared to control subjects. Moreover, the subset of "salt sensitive" hypertensives had even lower urinary excretion rate of irET-1, under well-controlled similar conditions. Furthermore, there seems to be a significant, albeit weak, negative correlation between MAP and urinary irET-1 levels when data from controls and patients were grouped. These observations may suggest that there is an inverse relationship between systemic arterial blood pressure and the rate of urinary excretion, but not plasma levels, of ET-1.

To understand the mechanism of the observed reduction in urinary irET-1 levels in hypertension the possible sources of urinary irET-1 should be discussed. These sources could be either circulating ET-1, which is filtered by the kidney, or local production within the kidney. Renal mesangial cells have been reported to contain mRNA for the precursor of ET-1 and to release ET-1 into the culture media [12]. Likewise, renal epithelial cells [13], have been shown to synthesize and release ET, and the renal medulla has been demonstrated to contain large amounts of irET [14]. On the other hand, the urinary clearance of ET-1 from the plasma, as determined by clearance studies of radioiodinated ET-1, is very low [15]. Thus, it is likely that urinary endothelin excretion reflects renal production of the peptide. Therefore, the decreased amounts of urinary irET-1 in patients with essential hypertension represent a genuine reduction in the rate of production of ET-1 within the kidney. This reduction in renal ET-1 synthesis may be secondary to systemic hypertension with high renal perfusion pressure, similar to the pressure-related renin release from the juxtaglomerular apparatus. However, the possibility that the decreased renal production of ET-1 is a primary phenomenon in these patients and has an etiologic role in the development of essential hypertension cannot be excluded. Renal impairment as a cause of the reduced excretion of ET-1 seems unlikely because renal disease is reportedly associated with an increase, rather than a decrease, in renal excretion of ET-1 [16].

The physiologic role of ET-1 in the kidney is still unknown. The renal medulla is rich in both irET and binding sites for ET [17], suggesting that ET may have an autocrine or paracrine action in the kidney [14]. In addition to well-recognized renal vascular effects, ET-1 also has glomerular [18], and renal tubular effects. Perhaps most importantly, it has been suggested that ET-1 has natriuretic properties [19, 20], probably by inhibition of tubular Na/K-ATPase activity. ET-1 also inhibits the vasopressin-induced accumulation of cyclic adenosine monophosphate in renal collecting ducts [21]. If ET-1 has a role in the renal handling of sodium and water, as suggested by these reports, then the low levels of ET-1 may have relevance to the secondary perturbations in renal sodium handling in patients with hypertension.

Although the low levels of urinary endothelin in hypertension were not anticipated, they seem to be in agreement with the reported decrease in renal medullary tissue content of irET-1 in experimental hypertension in animals [14, 22]. The markedly reduced urinary ET-1 levels in our hypertensive patients, the even lower levels in "salt sensitive" patients, and the low tissue levels of ET-1 in the renal medulla in experimental hypertension, suggest that the renal endothelin system has a role in the development and maintenance of clinical hypertension. In conclusion, patients with essential hypertension, particularly "salt sensitive" patients, have markedly reduced urinary levels, but normal plasma levels, of irET-1. There is a significant negative relation between renal excretion of irET-1 and mean arterial blood pressure. Further studies are needed to examine the possible causal relationship between the decreased renal production and excretion of ET-1 in hypertension and the pathogenesis of essential hypertension and salt sensitivity.

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